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综述

M2型丙酮酸激酶在肿瘤细胞中的多功能性

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摘要:丙酮酸激酶是葡萄糖代谢的关键限速酶。它主要有4种同工酶形式,L型、R型、M1型和M2型。M2型丙酮酸激酶主要表达于肿瘤细胞当中。肿瘤细胞对葡萄糖的代谢主要是通过有氧糖酵解的形式来完成,这一过程中M2型丙酮酸激酶起着关键限速酶的作用,同时在肿瘤细胞增殖过程中也起着调控信号转导的作用。M2型丙酮酸激酶为肿瘤细胞的增殖提供了能源和物质基础,它可以作为早期检测肿瘤的生物标记物。深入了解M2型丙酮酸激酶在肿瘤中的作用及机制可以为肿瘤靶向治疗提供新的思路。
关键词:M2型丙酮酸激酶;肿瘤;糖酵解;非糖酵解

Versatility of pyruvate kinase M2 in tumor cells

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Abstract: Pyruvate kinase is a critical rate limiting enzyme in glucose metabolism. It has mainly 4 isozyme forms: L type, R type, M1 type and M2 type. Pyruvate kinase M2 (PKM2) mainly expressed in tumor cells. The metabolism of glucose by tumor cells is mainly accomplished by aerobic glycolysis. During the process, PKM2 plays a key role in the rate limiting enzyme. It regulates the signal transduction in the process of tumor cell proliferation. Pyruvate kinase M2 provides energy and material basis for the proliferation of tumor cells. It can be used as a biomarker for early detection of tumor. The exploration of mechanism of PKM2 in tumor can provide a new way to tumor targeted treatment.
Keyword: M2 type pyruvate kinase; tumor; glycolysis; non glycolysis

细胞生存的关键因素是获得充足的能量供应。肿瘤细胞在低氧条件下利用糖酵解产能供给细胞生存,这种现象称为Warburg效应[1]。有研究证实M2型丙酮酸激酶(PKM2)是糖酵解的关键调节酶,为细胞增殖提供了核酸、氨基酸、脂类等物质基础[2]。除了具有糖酵解功能,PKM2的非糖酵解功能也引起了广泛的关注。本文主要描述PKM2在肿瘤细胞中的功能,以及探讨其潜在的治疗应用价值。

1 丙酮酸激酶的4个亚型

丙酮酸激酶是糖酵解酶,它可以催化磷酸烯醇式丙酮酸和ADP产生丙酮酸和ATP。丙酮酸激酶有4种亚型(PKL、PKR、PKM1和PKM2)。L型和R型被PKLR基因编码,它们的表达具有组织特异性。L型主要表达在肝、肾和肠组织中;R型主要表达在红细胞中[3]。PKM1和PKM2被PKM基因编码,是mRNA不同剪切体的产物,PKM1是外显子9,主要表达在成人的分化组织中,如脑组织和肌肉组织[4-5];PKM2是外显子10,主要表达在胚胎细胞、成人干细胞和肿瘤细胞中[6-7]。

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2 PKM2在肿瘤细胞中的表达

除了胚胎细胞和成人干细胞,PKM2是肿瘤细胞中主要的存在形式[8-9]。PKM2的表达在肿瘤生长过程中起着重要的作用[10-11]。近年来,有研究发现了PKM2表达的调节因子mTOR,mTOR的激活导致PKM2表达的增加,从而反转录激活低氧诱导因子(HIF-1),进而促进了肿瘤的发生。因此,PKM2是早期检测肿瘤的生物标志物。

3 PKM2的糖酵解功能

Warburg效应中PKM2是以低活性的酶形式存在的,这为肿瘤的发生发展提供了有利的条件。首先糖酵解途径产能的速度比氧化磷酸化途径更快[12],同时可以使含碳有机物更快速合成,从而为肿瘤细胞的发生发展提供大量的物质基础[13]。有研究证实,通过大量葡萄糖的消耗糖酵解能以较快的速度产生

ATP，但是产生ATP的量是比较低的[14]。其次低活性PKM2促进了糖酵解中间代谢产物核酸等的生成和堆积，这为肿瘤细胞的发展提供了物质基础[15-16]。总之，低活性PKM2促进了糖酵解，从而为肿瘤细胞增殖提供了各种资源，比如能量和物质。

PKM2有3种活性形式：无活性的单体，低活性的二聚体和高活性的四聚体，而PKM1主要以高活性四聚体形式存在[17]。肿瘤细胞PKM2主要以低活性的二聚体形式存在[18]，而正常的增殖细胞则以高活性的四聚体形式存在[8]。有研究报道PKM1表达的细胞中比PKM2表达的细胞中丙酮酸激酶的活性要高，这些细胞消耗的氧量更多，产生的乳酸少，对于线粒体ATP生成抑制剂寡霉素更加敏感[19-20]。肿瘤细胞中PKM2的丝氨酸和酪氨酸位点会发生磷酸化，PKM2上的磷酸酪氨酸能够促进1,6-二磷酸果糖从PKM2的结合口袋上释放出来，使得四聚体转变为无活性的二聚体，从而使糖酵解向生物合成转变[21]。使细胞有氧糖酵解能力增加，加快肿瘤的发展。E7蛋白是人乳头瘤病毒16型中的癌蛋白，和PKM2结合以后能加剧PKM2二聚化，加快肿瘤发展。

有研究报道，有多种因子可以调节PKM2二聚体和四聚体形式的转换[22-24]。例如，糖酵解中间代谢产物1,6-二磷酸果糖可以对PKM2进行变构调节。二聚体形式的PKM2可以被1,6-二磷酸果糖变构调节生成四聚体。PKM2上的磷酸酪氨酸能够促进1,6-二磷酸果糖从PKM2的结合口袋上释放出来，使得四聚体转变为无活性的二聚体，从而使糖酵解向生物合成转变。四聚体的PK与细胞的高ATP:ADP及GTP:GDP的比值有关，二聚体形式的则相反。丝氨酸产生于糖酵解中间代谢产物3-磷酸甘油酸，它也是PKM2的调节因子[25]。除此之外，癌基因蛋白HPV-16 E7和有活性的pp60^{V-Src}结合四聚体形式的PKM2后会产生二聚体形式的PKM2[8]。近年来研究发现高氧环境下会引起PKM2四聚体形式的解离，从而降低了PKM2的活性[26-27]，并且PKM2赖氨酸残基乙酰化抑制了PKM2酶活性，从而通过伴侣介导的自噬导致了降解[28]。

4 PKM2的非糖酵解功能

PKM2可以和很多分子相互作用[29-31]，其中有些分子影响了PKM2的糖酵解功能，这些分子直接调控Warburg效应。大量研究报道了PKM2的非糖酵解功能，特别是PKM2在转录中的作用引起了广泛的关注，有研究报道PKM2可以和HIF-1直接相互作用，从而促进HIF-1靶基因的转录激活[32]。也有很多研究报道了PKM2的核易位。白细胞介素-3和表皮生长因子受体激活，可以导致PKM2核易位的发生，从而激活基因转录和细胞增殖[33]。当细胞受到生长因子信号刺激时，PKM2活性受到抑制，加强糖酵解途径，导致葡萄糖代谢产物积累，为细胞增殖提供能量和物质基础。美国德克萨斯大学一研究团队也揭示PKM2可通过一种非代谢机制促进细胞增殖和肿瘤形成[34]，该研究证实，PKM2对表皮生长因子受体是十分重要的，提升β连环蛋白活性，从而引起基因表达、细胞生长及肿瘤的形成。该研究证实在人类癌细胞中表皮生长因子受体信号活化可诱导PKM2易位进入细胞核，在细胞核中PKM2的K433与β-catenin的c-Src磷酸化Y333位点结合，进而调控Cyclin D1表达，从而导致细胞增殖速度加快以及肿瘤的形成。

细胞核中的PKM2主要以二聚体的形式存在，然而细胞质中的PKM2以二聚体和四聚体两种形式存在[35]。除此以外，细胞核中的PKM2发挥着蛋白激酶的作用，它可以使Stat3磷酸化进而激活肿瘤相关基因的转录，比如Mek5[36]。综合这些研究可推断PKM2在促进肿瘤细胞发生发展过程中具有两方面的作用：首先，细胞质中二聚体形式的PKM2作为丙酮酸激酶发挥作用，低活性的PKM2维持了细胞的糖酵解过程，并且促进了糖酵解中间代谢产物的生成，为肿瘤细胞的增殖提供物质基础；其次，细胞质中二聚体形式的PKM2易位入核后在细胞核中起着蛋白激酶的作用。它可以磷酸化特殊的细胞核蛋白，进而促进基因转录，从而使肿瘤细胞增殖。近年来研究发现，PKM2在各种环境条件下具有不同的功能，PKM2与免疫反应、基因组不稳定性、血管再生、发病机制等相关[37]，这些疾病的发生是否与PKM2的糖酵解功能和非糖酵解功能相关需要进一步确定。

5 PKM2作为肿瘤治疗靶点的前景

PKM2在肿瘤细胞代谢和信号转导中起着很多重要的作用，PKM2被认为是肿瘤疾病治疗的理想靶点。RNA干扰和肽适体可以消融PKM2，并产生破坏肿瘤生长、诱导细胞凋亡、增加化疗药物敏感性等抗肿瘤效果[38]。PKM2的小分子抑制剂可以抑制糖酵解并引起细胞死亡。但是PKM2的靶向治疗到目前为止还是难点，一是PKM2除了在肿瘤组织中表达，它在正常增殖组织中也有表达，二是通过小干扰RNA对PKM2进行基因沉默不能完全抑制肿瘤细胞增殖。因此激活PKM2催化活性的复合物可能会作为肿瘤治疗的一个方式[39]。PKM2在正常组织中是以高活性形式存在的，而在肿瘤组织中则是以低活性形式存在。激活剂可以抑制肿瘤细胞糖酵解和细胞增殖，PKM2激活剂与1, 6-二磷酸果糖都可以诱导

PKM2四聚体形式形成。由于核PKM2是以二聚体形式存在的，PKM2激活剂能够阻止PKM2从细胞质进入细胞核，从而抑制了核PKM2的功能。换言之，PKM2激活剂可能会抑制PKM2糖酵解功能和非糖酵解功能。PKM2糖酵解功能抑制剂可以抑制肿瘤细胞糖酵解，导致了ROS生成量增加以及肿瘤细胞快速生长所需能源物质供应量的降低。PKM2非糖酵解功能抑制剂可以抑制肿瘤相关基因，如Mek5，c-Myc，和各种HIF-1靶基因的活性。它们在肿瘤治疗过程中的合理性需要进一步研究证实。

6 展望

PKM2的糖酵解功能和非糖酵解功能为肿瘤细胞的生长和生存提供了条件。在早期肿瘤形成过程中就有PKM2的表达，并且在肿瘤病人的血液和粪便中可以检测到[19]；进一步研究表明PKM2水平与肿瘤大小和肿瘤分期有关。

微环境可以引起肿瘤组织的代谢异质性，代谢异质性可能由每个肿瘤细胞不同的能源物质供给以及氧供给有关，这是由每个肿瘤细胞的定植部位离血管的距离不同造成的。PKM2通过糖酵解功能和非糖酵解功能为肿瘤细胞恶性表型的形成提供了条件，说明PKM2可能会成为肿瘤治疗的一个有效靶点。然而PKM2具有多面性，它在细胞内发挥的作用很多很复杂，而且以PKM2作为靶点进行肿瘤治疗时，在正常细胞产生的效果也很难评估。因此，在PKM2激活剂和抑制剂被用作干预治疗之前，需要进一步研究。

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